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SPACE SCIENCES LABORATORY

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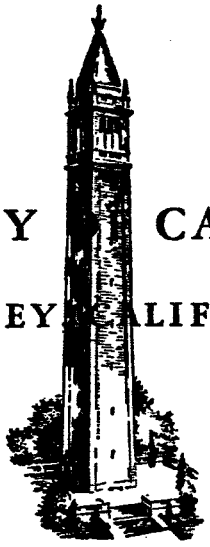

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Space Sciences Laboratory
University of California
Berkeley 4, California
Samuel Silver
Director

ANNUAL PROGRESS REPORT ON NASA

GRANT NSG-479 CHEMISTRY OF LIVING SYSTEMS

for period ending July 1, 1964

Reported by
Thomas H. Jukes
Faculty Investigator

July 1, 1964

I. Scientific Program

The scientific program of the Project entitled "Chemistry of Living Systems" had as its beginning a statement by Dr. Orr Reynolds regarding the objectives of the Space Biology Program. It was proposed that a fundamental program in biochemistry at the sub-cellular level would fit in with the National Aeronautics and Space Administration's objectives of searching for knowledge concerning the origin and evolution of life. The general field of molecular biology is expanding rapidly and is providing information on the basic mechanisms needed for the existence and continuation of life. It is evident in current discussions concerning Mars that the possibility of extraterrestrial life is best considered in the framework of the minimum requirements of biochemical systems that can reproduce, evolve and survive. This Project is concerned with an exploration of the fundamental nature of such systems as found in terrestrial organisms.

The main acitivities in this Project during 1963-1964 were obtaining new staff members, setting up and equipping laboratories, and starting laboratory work on various projects. Most of the funds were spent on laboratory equipment and facilities. We now have six laboratories of varying sizes in the north end of the University of California facilities at 1414 South 10th Street, Richmond, California. Five more laboratories, approximately 20' x 30' are

within 2 or 3 weeks of completion as of July 1, 1964. Various large pieces of modern equipment have been purchased including ultracentrifuges, spectrophotometers, fraction collectors, radioactive counters and an electrophoresis apparatus.

II. Projects

The following projects were initiated during the year:

1) Enzyme content and function of polysomes, Drs. I. D. Raacke, J. Fiala, Miss M. Krotkov and Mr. R. Tsai, (in collaboration with the Kaiser Laboratory of Comparative Biology).

A) Self-sufficiency of E. coli polysome in protein synthesis.

Naturally occurring polysomes of E. coli, isolated by a sucrose density gradient, were investigated in respect to their binding with enzymes employed in protein synthesis.

Non-specific adsorption of enzymes to the ribosomes should give a pattern identical with the distribution of total protein in the gradient, specific adsorption would result in a largely different pattern. The study was therefore concerned with gradient distribution of some enzymes evidently employed in protein synthesis, especially of phosphatases specific for ATP, GTP, CTP and UTP.

All of these phosphatases were found to be concentrated in the polysome fractions, which are generally considered as the active structures in protein synthesis: their distribution is therefore completely different from that of total protein. Heavy fractions display the highest specific activities of enzymes per mg protein, and in the same time contain the largest proportion of the total

incorporating activity of the extract.

In assays currently used for the amino acid incorporation, supernatant fraction is usually added to the system; added supernatant contains at least 1 mg of protein per 1 ml of incorporation mixture. In present study, incorporation assays were performed without addition of any supernatant; incorporation was performed in the presence or absence of various cofactors. It was found that the function of polysome fractions in complete absence of supernatant resulted in substantial incorporation of radioactive leucine or algal protein hydrolysate. Addition of sRNA caused only a moderate increase in incorporation. There was only partial requirement for ATP, but absolute requirement for a triphosphate-generating system.

These results suggest that the polysomes bind triphosphatases, some amount of sRNA and all the enzymes necessary for amino acid incorporation. The supernatant stimulates incorporation by polysomes in the absence of added sRNA; after addition of sRNA, the effect of supernatant varies from a small stimulation to a tenfold inhibition of activity. The effect is dependent on the ratio of supernatant protein to ribosomal protein, optimum ratio being about 0.2.

It is therefore evident that polysomes active in amino acid incorporation are optimally saturated with necessary enzymes. The stim-

ulation by supernatant is possibly due to its sRNA content.

B) XTPases in rabbit reticulocyte ribosomes.

To extend our findings about ribosome-bound XTPases in E. coli to the mammalian system, experiments are under way with polysomes prepared from rabbit reticulocytes. Crude extracts and once-and twice-pelleted ribosomes were prepared according to A. Rich, R. Schweet and M. Takanami (various publications) and patterns of ribosomal proteins distribution were studied in gradients under various conditions.

Ribosomal nucleoside triphosphatases were found in once-pelleted ribosomal fractions. The specific activity of ATPase was higher in the polysome region than in the 78 S ribosomal peak.

C) Publications

These are listed in section V. Several more manuscripts are being written.

2) Sequence of bases in RNA, Dr. S. Mandeles, H. Chung, F. Fearney, (additional support by N.I.H. grant starting May 1, 1964). The information contained in DNA and RNA is present as a sequence of the bases; in RNA these are adenine, cytosine, guanine and uracil. The determination of the order in which these bases are placed is a problem of much difficulty but of great importance. The best approach seems to be a combination of different procedures to split the RNA molecule and produce specific fragments that can be

analyzed. This work is being undertaken using tobacco mosaic virus RNA as the model compound and with a combination of enzymatic and organic chemical techniques. This is discussed in Appendix 1. The expenses for this grant are divided between the National Aeronautics and Space Administration and the National Institutes of Health.

Since September, 1963, work has been carried out in three areas subsidiary to the main problem of sequences in nucleic acids.

The first task was the production of large amounts of TMV from infected leaves. It was found that by selecting certain features from the procedures of Boedtker and Simmonds, and of Steere, we could increase by 100-fold the amount of leaves processed. We can now produce as much as 18 gm of TMV per man-day, in contrast to the 0.5 gm per man-day in the summer of 1963. The product obtained is essentially purer as judged by electron photomicrographs and gross appearance of suspensions. Highlights of our procedure are:

1) Infected leaves are homogenized in EDTA buffer and the homogenate is centrifuged in an International basket head.
2) The juice obtained is then mixed with celite and charcoal and filtered with suction.
3) The decolorized virus suspension is centrifuged at 19000 RPM in the Spinco for only 1.5 hours (85% of virus obtained, remaining virus requires at least 1.5 hours more).

4) The virus pellet is suspended in EDTA buffer and subjected to two cycles of differential centrifugation.

The next problem was the purification of T_1 ribonuclease for use as a specific hydrolytic agent. The source of T_1 is the Japanese brand of Takadiastase (Aspergillus oryzae) powder. The particular batch of Takadiastase sent to us contained a cellulase that made the use of dialysis in cellophane sacs impractical and seriously decreased the efficiency of DEAE-cellulose columns for purifying the ribonuclease. We found that this cellulase could be removed by phenol extraction and acetone precipitation, followed by passage through a carboxymethyl-Sephadex column. This treatment apparently also markedly diminished the content of contaminating phosphomonoesterase and phosphodiesterase.

When purified T_1 ribonuclease was obtained, we expressed its activity in terms of the number of microequivalents of phosphodiester bond broken per mg of enzyme per minute at 30° (using a titrimetric method). Heretofore, the only assay methods for T_1 ribonuclease depended on the increase in the UV absorption of the acid-soluble fraction of nucleic acid in the presence of enzyme. We carried out a comparative study relating the values obtained by the titrimetric method to those obtained from the spectrophotometric methods. For our purposes, the titrimetric method is more meaningful because we need to know the extent of hydrolysis in terms of the number of

bonds broken. A report embodying these results has been submitted for publication.

A third problem was the separation of nucleic acid oligomers and the determination of their base composition. During the period of time before T_1 ribonuclease was purified, we had been using pancreatic ribonuclease and achieving satisfactory separation of nucleic acid oligomers according to chain length with the Tomlinson-Tener column (DEAE-cellulose in 7M urea). Separation of oligomers from hydrolysates of homopolymers such as poly-A were also successful on this type of column. However, when T_1 ribonuclease hydrolysates of YRNA* were chromatographed under identical conditions, separations of oligomers only up to the 5-mer was observed. Altering the various parameters of chromatography such as the gradients of pH and ionic strength, provided no improvement and usually resulted in a loss of resolution. These observations confirmed similar observations made at N.I.H. by Sober et. al. This group has also published a report recently describing the use of DEAE-Sephadex instead of DEAE-cellulose for chromatography of oligomers. A study of this system is now underway.

Paper electrophoresis-chromatography and thin layer chromatography are being investigated for application to the determination of base composition. Presently it appears as if thin layer chromatography (TLC) will be satisfactory for use in base composition analysis.

*yeast RNA

3) RNA polymerase, Dr. J. Krakow, Miss L. Holmes, Mr. W. Horsley. Hereditary information in DNA is transcribed into RNA by the enzyme RNA polymerase. There is general agreement that all three forms of RNA; messenger, transfer and ribosomal; are produced by this enzyme and that only one of the two strands of DNA is transcribed into RNA.

A) Inhibition of RNA polymerase by acid dyes.

We have found that RNA polymerase obtained from Azotobacter vinelandii is inhibited by acid dyes. Of the several tested the sulfonic acid Azo dye, Congo red and the tetrabromo derivitive of fluorescein, Eosin Y, are the most potent. At a concentration of 1×10^{-6} M the native DNA-directed synthesis of RNA is inhibited 70% by Congo red and 50% by Eosin Y. With methyl orange, a dye related to Congo red, a 50% inhibition is obtained at 10^{-4} M indicating some degree of specificity for inhibition. The very specific and potent inhibitor of RNA polymerase, Actinomycin D, at 2×10^{-7} M, results in a 70% inhibition of RNA synthesis in vitro when native DNA is used as template. Actinomycin D acts by binding to d-GMP residues in the DNA helix. It is without effect when RNA polymerase is primed by polyribonucleotides and is much less effective with denatured DNA. The inhibition of RNA polymerase by Congo red is independent of the nature of the primer and we believe that these dyes act by binding to the enzyme. Both Congo red and Eosin Y are thought to interact with proteins by hydrogen bonding. Since RNA

polymerase probably interacts with the primer in this fashion it is possible that these dyes are blocking the primer site, although any alteration in the tertiary structure of the enzyme could also result in inhibition.

Further studies have shown that the poly A polymerase found in E. coli ribosomes is also sensitive to Congo red; 4×10^{-6} M dye gives a 75% inhibition of AMP incorporation. This concentration of dye unexpectedly results in a slight (30%) stimulation of poly-U-directed polyphenylalanine synthesis in the E. coli ribosome-supernatant system. At 10^{-4} M, 90% inhibition of phenylalanine incorporation is obtained. The stimulation of polypeptide synthesis at the lower dye concentration may be causally related to inhibition of poly A synthesis, since the poly A formed at the ribosome may tend to complex with the poly U and inhibit polyphenylalanine formation.

B) Investigations of the mechanism of action of polyamines in stimulating RNA polymerase.

When RNA is synthesized by RNA polymerase with native calf thymus DNA in the absence of polyamines, the product is 20% retained (at neutral pH) by Millipore filters. When polyamines are included, 80% of the RNA is retained. By using H^3 labelled T_2-DNA as the primer it is possible to show that DNA is also retained. According

to Nygaard and Hall (BBRC 12, 98 (1963)) from 10 to 50% of native phage DNA is bound by S & S membrane filters (0.4 μ pore size). The H^3-DNA used here shows 80% retention on S & S filters as well as on Millipore. This is not due to the presence of denatured DNA, which is 100% retained by these filters,nor is there any effect of polyamines in the absence of RNA synthesis. The high proportion of DNA retained is unfortunate from the standpoint of our work and more will be prepared and filterable DNA will be purified. In keeping with the results earlier shown with calf thymus DNA, RNA synthesized in the presence of spermidine is retained by the filters, and there is almost 100% retention of the DNA. This ability to retain the polymerase complex is shown by Millipore filters with pore size of 0.45 μ and 0.65 μ and SS membrane filters with pore size of 0.4 μ. As mentioned above only a small percentage of the RNA synthesized with calf thymus DNA as primer in the absence of polyamines is retained by the filters, with H^3T_2 DNA 60% of the RNA formed is retained (addition of spermidine after the reaction mix is chilled to 0^o results in 90% retention; there is no increase with calf thymus DNA directed product). Heating the complex in the presence of Na lauryl sulfate results in no loss of acid precipitable counts but dissociates the RNA from the complex (both with or without polyamines) and renders 90% of the RNA and 50% of the DNA filterable. This is in keeping with the findings of Bremer and Konrad (PNAS 51,

801 (1964)), who showed that the DNA-polymerase-RNA complex demonstrated by sucrose density centrifugation was dissociated by treatment with detergent.

Attempts to study these complexes by this method have not been successful due to poor recovery of C^{14} RNA. However, our results to date have indicated that the complex formed in presence of spermidine has an appreciably higher sedimentation rate than that formed in its absence. Work is continuing in this area to improve the procedure.

C) Studies on the Nature of the Time course for Native DNA directed RNA synthesis.

Regardless of the source of the enzyme, RNA polymerase primed by native DNA shows an initial rapid and linear rate of RNA synthesis with a subsequent plateau. We have shown that synthetic polyribonucleotides are effective inhibitors of RNA polymerase; this inhibition is probably due to competition for the template site on the enzyme. We have proposed that the RNA synthesized during the course of the reaction behaves in a manner similar to the polyribonucleotides and that product inhibition ensues. In order to test this hypothesis we carried out experiments where product RNA accumulation is minimal; i.e. in the presence of pancreatic ribonuclease (RNAase). With the amount chosen (50 μg RNAase per 0.25 ml reaction volume) less than 10% of the product was acid-insoluble. To measure enzyme

activity in the presence of RNAase the procedure was altered to detect release of P^{32} pyrophosphate from βγ labelled P^{32} ATP. It was found that the kinetics were virtually identical whether RNAase was present or absent. In addition the presence of RNAase apparently abolished the stimulation elicited by polyamines. These results are difficult to reconcile with the proposal of product inhibition, however, since RNAase leaves purine tracts intact we will extend these experiments to see whether concomitant addition of a ribonuclease (T_1 RNAase) which will further degrade the residual product will have any effect on the kinetics. We will also try to characterize the residual 10% of the product which is RNAase resistant to determine its base ratio and physical structure (since RNA-DNA and RNA-RNA complexes are resistent to RNAase.)

4) Studies of the composition and function of the chromosome of Bacillus subtilis, Dr. H. Yoshikawa, Dr. N. Kieffer, Mrs. B. Benjamin.

A) Storage of competent cells for transformation

It has been difficult to keep a competent B. subtilis cell for transformation. It was found that a mutant of B. subtilis strain 168, (leu^-, met^-, ade^- #27), was kept in a competent state for transformation when it was frozen in liquid nitrogen in the presence of 20% glycerol. The efficiency of transformation of the frozen cell was 80% at 7th day and 75% at 20th day of original efficiency.

B) Isolation and sulfanilamide resistant mutants and their analysis.

Mutants of W168, a wild type of B. subtilis, which are resistant to various concentrations of sulfanilamide were isolated by spontaneous mutation and selection. The analysis of the genetic structure of these mutants is being undertaken by transformation studies. A possibility of episomic nature of SA^{Y} gene is also being examined by acriflavine and proflavine treatment.

C) Isolation of an intact chromosome from B. subtilis.

Attempts were made to isolate an intact chromosome from B. subtilis. A thymine-requiring mutant of B. subtilis W23 was labelled with H^{3}-thymidine. Cells heated at 60^{o} C were lysed with lysozyme in 25% sucrose in a dialysis bag. The protoplasts thus obtained were dialysed against 0.5% duponol in 25% sucrose solution, for 12 hours without stirring. Any treatment which might cause shearing force to the chromosome was carefully avoided throughout the isolation process. A clean lysate was then centrifuged in a gradient of sucrose solution. It was found by this procedure that a chromosome identified by 3H thymidine peak is separated from both ribosome peaks (100S and 70S), and DNA of 20×10^{6} M.W. purified by the regular method. From the peak position in the centrifuge tube the sedimentation constant of the chromosome is estimated roughly as 200 or 300 S which suggests that the isolated chromosome is fairly large in size, probably a whole intact chromosome.

The following questions will be examined in this system:

1) Are there any materials other than DNA in the chromosome?

   a. Protein, especially basic protein?

   b. RNA, especially messenger RNA?

2) Does the size of the chromosome vary in different growth conditions? For example, a chromosome which has 4 forks should have 3 times higher M.W. than normal chromosome.

3) Can any cytoplasmic gene (or DNA) be separated from the chromosome by this method?

(5) Viral control of host genome

Dr. H. Kammen, Mr. R. del Vecchio. Work in this field was started in June, 1964. There is nothing to report as yet.

(6) Polypeptide formation of polysomes; Interaction of ribosomes with messenger RNA and transfer RNA.

Dr. M. Takanami will start work in this field in July, 1964.

(7) Studies On The Mechanism Of Antibody Production

Dr. David Freifelder, Mr. John Polacheck

(Cooperative studies at Donner Laboratory of Medical Physics)

NSG-479

Lymphocytes isolated from peripheral blood of humans possess the ability to make specific antibody in response to a particular antigen to which the subject is sensitive. Furthermore, they are capable of recognizing lymphocytes of another person as foreign and respond by the production of antibody. This recognition of foreign cells has been correlated with the rapidity with which skin grafts are rejected and is important to the general problem of transplantation immunity. An understanding of the mechanism of stimulation of antibody production by foreign cells may give valuable information concerning the rejection of homotransplants. Studies of this problem are being carried out in the Donner Laboratory at the University of California in collaboration with Drs. Albert Rubin and Kurt Stenzel of the Cornell University Medical College, New York City.

Considerable effort has been devoted to understanding the properties of the system which affect reproducibility and to methods for increasing the sensitivity of the assay. After several months of using morphological transformation as an indication of stimulus, it has been shown that a more reliable measure is to follow incorporation of isotopically labeled biochemicals into protein, ribonucleic acid, or deoxyribonucleic acid, all of which are equally

useful.

Using this technique we have to date shown that (1) recognized cells can be boiled without loss of stimulating activity; (2) there is a component in the system which antagonizes the response (this may be extremely important as a possible technique for counteracting the rejection response); (3) osmotically shocked cells can stimulate; (4) the activity of osmotic shockates resides mainly in particulate fractions.

Current efforts are devoted to fractionation of cells and the properties of the inhibiting reaction.

III. Laboratories

Five new laboratories and other auxiliary rooms were added in the Spring of 1964. The modifications of the existing building were financed by NSG-479. The laboratories have excellent facilities including hoods, benches, and the usual services. The auxiliary rooms include 3 offices (10' x 10') 5 incubator and instrument rooms and a walk-in refrigerator and freezer (10' x 20'). Four of the large laboratories will be occupied by staff members and the fifth will be used for large equipment.

IV Publications and Manuscripts

(a) Published 1963-64

1. Polyribosome-Bound Nucleoside Triphosphatases in Escherichia Coli. I. D. Raacke, J. Fiala, (Proc. Natl. Acad. Sci. 51, 2, 323-329 (1964).

2. Self-Sufficiency of Natural E. Coli Polysomes for Amino Acid Incorporation. I. D. Raacke, J. Fiala & T. H. Jukes, (Federation Proceedings, 23, 2, March-April (1964)).

3. Some Recent Advances in Studies of the Transcription of the Genetic Message. T. H. Jukes, (Advances in Biological and Medical Physics, vol. 9, pg. 1, Academic Press (1963)).

(b) In Press

1. Coding Triplets In The Evolution of Hemoglobin and Cytochrome-C. T. H. Jukes, (The Origin of Prebiological Systems, Academic Press).

(c) Submitted For Publication

1. Modifications In The Preparation And Assay Of T_1 Ribonuclease. H. Chung and S. Mandeles. Sent to Biochim. Biophys. Acta

2. Differential Distribution of RNA and Protein in the Ribosomal Fractions of E. coli. Sent to Biochim. Biophys. Acta. I. D. Raacke.

3. Specific Nucleoside Triphosphatases in Crude Extracts of E. coli. I. D. Raacke. Sent to Biochim. Biophys. Acta.

4. Fate of Nucleoside Triphosphatases on Purification of the Ribosomal System from E. coli. I. D. Raacke and J. Fiala. Sent to Biochim. Biophys. Acta.

5. Self-Sufficiency for Amino Acid Incorporation of Natural E. coli Polysomes. J. Fiala and I. D. Raacke. Sent to Biochim. Biophys. Acta.

6. The Effect of Pre-Incubation of Crude E. coli Extracts on the Distribution of Different Enzymes. I. D. Raacke. Sent to Science.

7. Ribonucleoside Triphosphatases in Ribosomal and Supernatant Fractions of Rabbit Reticulocytes. J. Fiala, S. Matsushita, M. Krotkov and I. D. Raacke. For Biochem. Biophys. Res. Comm.

(d) Other Recent Publications By Staff Members

1. Ribonucleic Acid Polymerase of Azotobacter Vinelandii, I. Priming By Polyribonucleotides. Joseph Krakow & Severo Ochoa. (Proc. Natl. Acad. Sci., 49, 1, 88-94 (January 1963).

2. Ribonucleic Acid Polymerase of Azotobacter Vinelandii, II. Formation of DNA-RNA Hybrids with Single-Stranded DNA as Primer. R. Warner, H. Samuels, M. Abbott, J. Krakow. (Proc. Natl. Acad. Sci. 49, 4 533-538 (1963)).

3. Ribonucleic Acid Nucleotidyl Transferase of Azotobacter Vinelandii. Joseph Krakow & Severo Ochoa. (Biochem. Zeit. 338, 796-808 (1963)).

4. Ribonucleic Acid Polymerase of Azotobacter Vinelandii. III. Effect of Polyamines. J. Krakow. (Biochim. Biophys. Acta 72, 566-571 (1963)).

5. A General Method for Determination of Nucleotide Sequences in Nucleic Acids. Stanley Mandeles & Ignacio Tinoco, Jr. (Biopolymers 1, 183-190 (1963)).

6. Transfer of Amino Acids from Soluble Ribonucleic Acid to Ribosome. Mituru Takanami & Kin-Ichiro Miura. (Biochim. Biophys. Acta, 72, 237-242 (1963)).

7. Interaction of Ribosomes and Synthetic Polyribonucleotides. M. Takanami & Toshio Okamoto. (J. Mol. Biol. 7, 323-333 (1963)).

8. Interaction of Ribosomes and Polydeoxyribonucleotides. Mituru Takanami and Toshio Okamoto. (Biochem. and Biophys. Res. Comm. 13, 4 (1963)).

9. Interaction of Ribosomes and Natural Polyribonucleotides. Toshio Okamoto and Mituru Takanami. (Biochim. Biophys. Acta 76, 266-274 (1963)).

10. An Estimate of the Size of the Ribosomal Site for Messenger RNA Binding. M. Takanami and G. Zubay. (Proc. Natl. Acad. Sci., 51, 6, 834 (1964)).

11. Sequential Replication of Bacillus Subtilis Chromosome , I. Comparison of Marker Frequencies in Exponential and Stationary Growth Phases. H. Yoshikawa and N. Sueoka. (Proc. Natl. Acad. Sci. 49, 559-566 (1963)).

12. Sequential Replication of the Bacillus Subtilis Chromosome, II. Isotopic Transfer Experiments. H. Yoshikawa and N. Sueoka. (Proc. Natl. Acad. Sci., 49, 6, 806 (1963)).

V. Laboratory Personnel

Dr. Jiri Fiala, Assistant Research Biochemist

Dr. Harold Kammen, Assistant Research Biochemist

Dr. Nathan Kieffer, U.S.P.H.S. Postdoctoral Fellow, Dept. of Genetics

Dr. Joseph Krakow, Associate Research Biochemist

Dr. Stanley Mandeles, Assistant Research Biochemist

Dr. I. D. Raacke, Associate Specialist Biochemist

Dr. Mituru Takanami, Assistant Research Biochemist

Dr. Hiroshi Yoshikawa, Assistant Research Biochemist

Mrs. B. Benjamin, Laboratory Assistant I

Mr. H. Chung, Laboratory Assistant I

Mr. R. del Vecchio, N.I.H. Training Grantee

Mr. F. Fearney, Laboratory Technician II

Miss L. Holmes, Laboratory Technician I

Mr. W. Horsley, Research Assistant I

Miss M. Krotkov, Post Graduate Research Biochemist II

Mr. J. Polacheck, Laboratory Technician I

Mr. R. Tsai, Laboratory Technician I

Appendix I. United States Department of Health, Education and Welfare, Public Health Service Research Grant #GM 1215801 was awarded May 6, 1964, to the University of California.

Principal Investigator: Thomas H. Jukes, Space Sciences Laboratory

Project Director: Stanley Mandeles, Space Sciences Laboratory

Associate Project Director: Ignacio Tinoco,Jr., Chemistry Department, University of California, Berkeley

Research Plan: Several methods have been proposed for the determination of the total nucleotide sequence in nucleic acids. These methods include:

1) Electron microscopy, where an extended nucleic acid polymer, suitably labeled to identify specific bases, is examined with a high resolution electron microscope. The sequence is determined from measurements of the relative position of the individual markers. An example of this method can be found in Beer and Moudrianakis (1962).

2) Step-wise degradation, where the nucleic acid is degraded to yield an identifiable base, nucleoside, or nucleotide from one end of the molecule and the remainder of the polymer. This procedure is applied in a cyclic fashion until the entire nucleotide sequence in the polymer is determined. An example of this approach has been proposed by Yu and Zamecnik (1960).

3) Overlap method, where nucleic acid is degraded partially in at least two different ways to yield oligomers. The sequence of nucleotides in each oligomer is determined and the data from one

experiment are compared with those from the other for reasons of overlap. Separate partial degradations are performed until the regions of overlap coalesce into a unique sequence. Examples of this approach may be found in reports by Madison et al (1963), and Rushizky et al (1962).

A number of other methods have been proposed for the determination of partial sequences in nucleic acids. One example of these is genetic mapping, where natural and chemically evoked mutational changes in bacteriophage are used to locate and in some cases identify the nucleotide base in DNA responsible for these changes. Champe and Benzer (1962) have reported considerable progress in mapping the rII region of T_4 bacteriophage DNA as well as the corresponding sites in the rII messenger RNA.

While we plan on using any method which seems suitable for a particular problem in sequence determination, we are presently using a procedure that is related to 2. and 3. above but which does not require step-wise degradation nor does it involve overlapping sequences. A description of this proposal has been published (Mandeles and Tinoco (1963)).

Briefly, the process involves the chemical modification of one end of the molecule (Ralph et al (1962), Monier et al (1960)), followed by partial enzymatic degradation and separation according to chain length of the modified polymers. A second reagent or handle

is determined. In this manner, the original nucleotide sequence can be reassembled in terms of sequential oligomeric fragments. The functions of the first chemically modifying derivative or handle are:

a) to serve as a reference point which permits each base to be positioned according to its distance from the modified end,

b) to provide a convenient means of separating oligomers with handles from those without handles,

c) to permit the use of additional parameters of fractionation of oligomers with handles from each other.

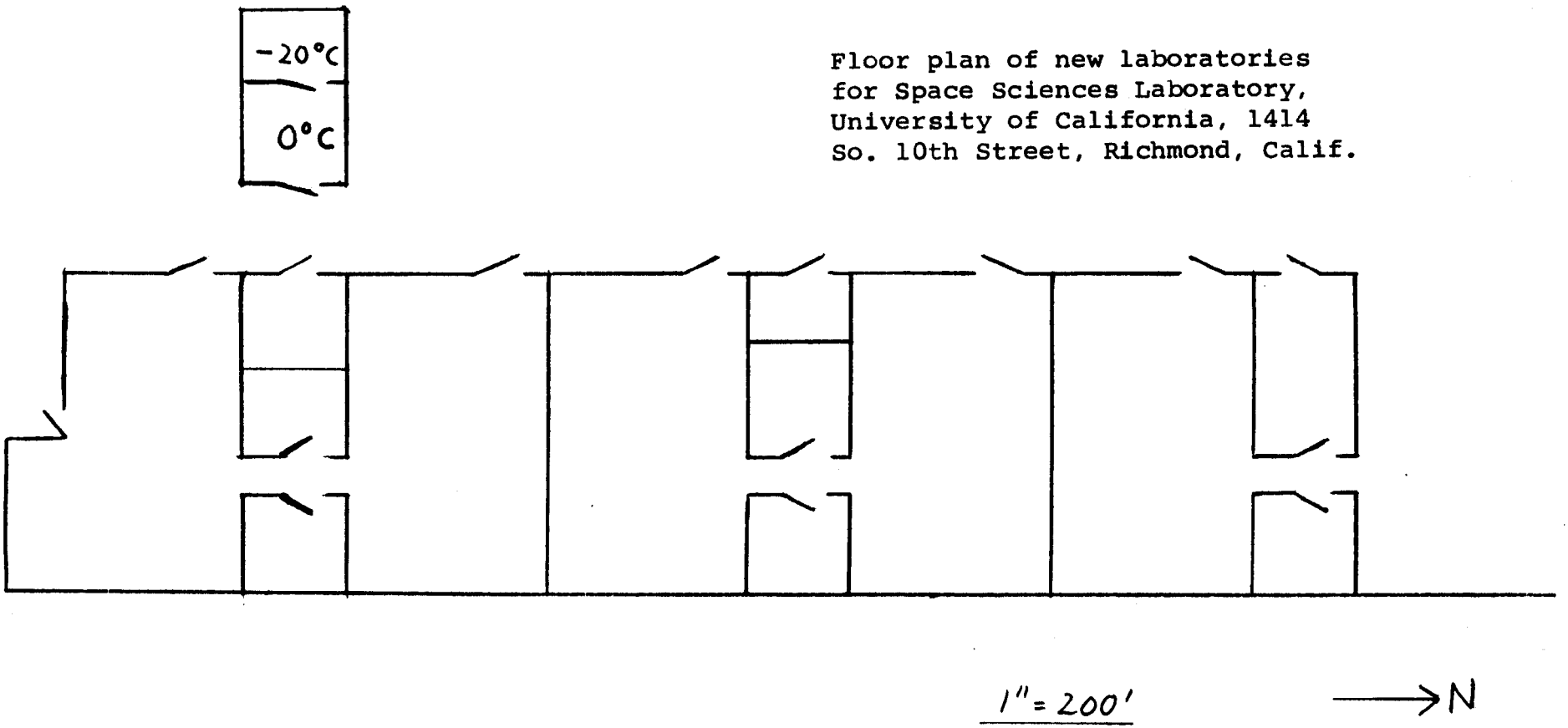
The purpose of the second handle and complete enzyme degradation step is to facilitate the selection of the end oligomer fragment in each fraction that is sequentially related to the end oligomer fragment in other fractions.

Work is now in progress on the use of this method for the determination of nucleotide sequences in TMV-RNA. This material was chosen because it presumably represents messenger nucleic acid and its sequence is related to the genetic code. (On the basis of a triplet code, only 8% of the TMV-RNA corresponds to a known protein; the remainder could code for other proteins, probably enzymes, and possibly other functions). In addition, TMV-RNA can be obtained in a form pure enough for use in determination of nucleotide sequences. In particular, the 3'and 5' linked ends have been identified as adenosine by Sugiyama and Fraenkel-Conrat (1961), and Sugiyama and Fraenkel-Conrat (1963).

Total Grant May 1, 1964-April 30, 1965 - $ 51,428.00

Appendix II Electron Microscopy of Single-Stranded DNA

During the Spring of 1964, Mr. John Polacheck's salary was paid by NSG-479 while he worked under the supervision of Dr. David Freifelder. He assisted in an investigation of the circularity of the infective form of Øx-174 DNA, and acknowledgement of this assistance by the National Aeronautics and Space Administration was made in the manuscript "Electron Microscopy of Single-Stranded DNA: Circularity of ØX-174 DNA" by David Freifelder, Albrecht K. Kleinschmidt and Robert L. Sinsheimer, which Dr. Freifelder has submitted to Science.



Floor plan of new laboratories for Space Sciences Laboratory, University of California, 1414 So. 10th Street, Richmond, Calif.